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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Examiner	:	
Group	:	185
Applicants	:	Sherie L. Morrison, et al.
Serial No.	:	07/675,106
Filed	:	March 25, 1991
For	:	RECEPTORS BY DNA SPLICING AND EXPRESSION

New York, New York
May 15, 1991

RECEIVED GROUP 180
MAY 23 1991

Hon. Commissioner of Patents
and Trademarks
Washington, D.C. 20231

SUPPLEMENTAL PRELIMINARY AMENDMENT AND
STATEMENT UNDER 37 C.F.R. §§ 1.56 AND 1.97

Sir:

Preliminary to substantive examination in the above identified application, kindly amend the application as follows:

IN THE CLAIMS

39. (Amended) A method for producing a receptor having two subunits, which comprises the steps of:

(a) transfecting a mammalian cell with a first DNA sequence coding for a first [one of the] subunit[s] of the receptor;

(b) transfecting the cell with a second DNA sequence, said second DNA sequence coding for a second [another of the] subunit[s] of the receptor, said second subunit being a subunit other than the first subunit; and

(c) maintaining the cell in a nutrient medium, so that [whereby] the cell expresses the first and second DNA sequences and the resultant subunits are intracellularly bound together to form a receptor.

45. (Amended) A method as recited in claim 43 wherein the cell does not endogenously produce any [endogenous] immunoglobulin chains.

50. (Amended) A receptor as recited in claim 49, said receptor being [comprising a molecule] selected from the [following] group consisting of major histocompatibility complex class I, major histocompatibility complex class II, antibody, T cell receptor and CD8.

51. (Amended) A receptor as recited in claim 49 wherein the receptor is a chimeric antibody having a variable region substantially the same as that found in a first mammalian source and having a constant region substantially the same as that found in a second mammalian source, said second mammalian source being from a mammalian species other than that of the first mammalian source.

Please add the following claims:

52. A method as recited in claim 45 wherein the cell is a murine myeloma cell.

53. A method as recited in claim 47 wherein the cell is murine myeloma cell.

54. A method for producing a receptor having two subunits, which comprises the steps of:

(a) transfecting a mammalian cell with a plasmid comprising a first DNA sequence coding for a first subunit of the receptor and a second DNA sequence coding for a second subunit of the receptor, said second subunit being a subunit other than the first subunit; and

(b) maintaining the cell in a nutrient medium so that the cell expresses said first DNA sequence and said second DNA sequence and the resultant subunits are intracellularly bound together to form a receptor.

REMARKS

Applicants have amended the claims to more particularly recite the subject matter of the present invention. Support for added claims 52 and 53 is provided in the specification at page 17, line 26 - page 19, line 27. Added claim 54 is supported at page 11, lines 15-18 of the specification. Support for the amendments to claims 39 and 51 may be found in the specification at page 2, line 20 - page 4, line 22. None of these amendments or additions to the claims constitutes new matter.

This application is a file wrapper continuation of United States patent application 441,189, filed November 22, 1989. In a Preliminary Amendment filed concurrently with this file wrapper continuation application on March 25, 1991, applicants cancelled the prior pending claims (claims 14-35) and added claims 39-51. The claims now pending in this application are directed to methods for producing a receptor having multiple subunits and to the products of those methods. These claims are novel and non-obvious in view of the documents cited herein, as well as those of record in the parent applications.

A receptor is a molecule that specifically binds a defined molecular structure. Examples of receptors include an antibody which interacts with a specific antigen, a T cell receptor, and a major histocompatibility complex antigen. The present invention relates to the production of receptors having multiple subunits by transfecting into and co-expressing in a mammalian cell multiple exogenous DNA constructs, each coding for one of the receptor subunits.

Two illustrative embodiments of this invention are disclosed in the present application. In the first embodiment, DNA constructs coding for chimeric mouse-human kappa light and gamma heavy chains of phosphocholine binding

antibody were transfected and co-expressed in the J558L cell line. J558L is a heavy chain loss variant mouse myeloma cell line. Phosphocholine binding antibodies were produced in the J558L cells according to the methods of the invention.

In the second embodiment, the same DNA constructs coding for chimeric mouse-human kappa light and gamma heavy chains of phosphocholine binding antibody were transfected and co-expressed in the P_3 cell line. P_3 is a non-immunoglobulin producing mouse myeloma cell line. Phosphocholine binding antibodies were produced by the P_3 cell line following the methods of this invention.

The present invention is patentable over the documents of record in this application for several reasons.

Prior to the present invention, transfection of a single exogenous DNA sequence coding for one chain of an immunoglobulin into a mammalian cell which also expresses endogenous DNA coding for another immunoglobulin chain had been described. Gillies et al., Cell, 33, pp. 717-28 (1983); Oi et al., Proc. Natl. Acad. Sci. USA, 80, pp. 825-29 (1983); Rice et al., Proc. Natl. Acad. Sci. USA, 79, pp. 7862-65 (1983). The endogenous-exogenous approach followed in each of these documents produced tetramers which were not functional receptors capable of antigen binding because the binding sites of the exogenous immunoglobulin chains were not complimentary to the binding sites of the endogenous chains.

Specifically, Gillies transfected and expressed an exogenous heavy chain gene in J558L, a heavy chain loss mouse myeloma cell. The heavy chain formed a tetramer with the endogenous lambda light chain. Oi transfected and expressed an exogenous kappa light chain gene in both J558L and in 27-44, an immunoglobulin producing mouse hybridoma

cell. The exogenous kappa chain was secreted by the hybridoma cell in assembly with the endogenous gamma heavy chain. Rice transfected a light chain loss variant of a murine myeloma cell with a kappa light chain gene. The exogenous light chain assembled with the endogenous gamma heavy chain.

Prior to the present invention, however, it was believed that the endogenous-exogenous approach would not always produce an immunoglobulin, let alone one that binds an antigen. In Oi, a mouse myeloma cell supported expression of a transfected immunoglobulin gene. However, a rat myeloma cell line which synthesizes and secretes a rat kappa light chain, when treated in the same manner as the mouse myeloma, did not support expression of a transfected immunoglobulin chain gene, even though the cell did produce an endogenous light chain.

In sum, prior to the present invention, the art as a whole taught that a cell that expresses an endogenous immunoglobulin chain gene would not necessarily express a transfected exogenous immunoglobulin chain gene. Thus, it would not have been obvious that a cell line that expresses one exogenous gene, such as J558L, would express a second exogenous gene simultaneously. Likewise, it would not have been obvious that a non-immunoglobulin producing myeloma cell line, such as P_3, would express one transfected immunoglobulin gene, let alone two of them.

Other documents cited by the Examiner in the parent applications refer to co-transfection and co-expression of immunoglobulin chain genes. See Cabilly et al., United States patent 4,816,567 ("Cabilly patent"); Cabilly et al., European patent application 125,023; and Cabilly et al., Proc. Natl. Acad. Sci. USA, 81, pp. 3273-77

(1984). These documents, however, do not enable the production of receptors in mammalian cells.

The Cabilly documents refer to experimental work in which bacterial cells were co-transformed with heavy and light chain antibody genes. The double transformants expressed those heavy and light chain genes. For example, the Cabilly patent states that an extremely small portion of these chains --0.76%-- then "recombined" to form anti-CEA antibody.

In discussing this low degree of recombination in a declaration submitted during prosecution of the application which issued as the Cabilly patent, one of its inventors explained that the recombination that occurs after heavy and light chains are co-expressed in a bacterial cell is essentially the same as the recombination that occurs after the heavy and light chains are expressed in separate bacterial cells. In the words of the declaration, "[a]s expected, the results with the co-transformant extracts and combined extracts were essentially the same..." (Wetzel Aff., p.2, July 22, 1986, submitted in support of Cabilly's July 24, 1986 Amendment -- copy attached hereto).

Applicants' invention is patentably distinct from the bacterial work described in the Cabilly documents, because it permits the production of properly assembled receptors by mammalian cells, rather than the reconstitution of tetramers from peptide chains produced by bacterial cells. Applicants create vectors containing genes for immunoglobulin heavy or light chains. Applicants then transform mammalian cells with those vectors. Those transformed cells produce receptors that exhibit binding specificity for the desired antigen. Expression of these functional receptors occurs by virtue of the mammalian cells' ability to glycosylate and fold the peptide chains

that they produce, thereby providing those chains with the appropriate tertiary structure.

None of those processes occur in the bacterial cell expression system of Cabilly. Bacterial cells lack the endoplasmic reticulum found in mammalian cells and which plays a key role in processing and folding the peptide chains produced in those cells. No glycosylation occurs in bacteria and the lack of a processing mechanism in bacterial cells results in a three-dimensional structure that is not capable of binding with antigen. Furthermore, Cabilly's reconstitution and reconstruction of an extremely low level of "antibody" (0.76%) does not reach the level of antibody expression achieved by the present invention. Thus, applicants' receptors and methods for their production are patentably distinct from Cabilly's production of immunoglobulin peptides in bacterial cell systems.

Those skilled in the art have recognized the failure of the Cabilly approach to succeed in producing properly folded and assembled immunoglobulins. Arne Skerra and Andreas Pluckthun comment on attempts to express immunoglobulins according to Cabilly:

> "Despite numerous investigations, the expression of functional whole antibodies or functional antigen-binding fragments of antibodies has not been reported for any bacterial expression system, and the chance of designing such an expression system has been viewed pessimistically[]....The purification of active antibodies or antibody fragments from yeast or any other microorganism has not been reported. In E. coli, the antibody protein could be produced only in a non-native state[], and refolding experiments led to only a small percentage of correctly folded recombinant antibodies. Moreover, it is difficult to purify the native protein from non-native contaminants, which complicates accurate measurements of binding constants, folding yields, and spectral properties." Science, 240, pp. 1038-1041 at p. 1038 (May 20, 1988) (copy attached hereto).

In that same issue of Science, Better et al. (pp. 1041-43 at p. 1041) state that "Escherichia coli has been

used to produce individual immunoglobulin chains internally that are not properly folded ..." Better cites to Cabilly's 1984 PNAS paper, supra p.5, and Boss et al., Nucleic Acids Research, 12, pp. 3791-3806 (1984) (copy attached hereto) for support. Likewise, Antonino Cattaneo and Michael S. Neuberger report that "the introduction of vectors driving expression of immunoglobulin cDNAs into yeast or Escherichia coli hosts has not resulted in effective antibody production--problems being encountered both with efficient assembly and with secretion (Cabilly et al., 1983; Boss et al., 1984; Wood et al., 1985)." EMBO J., 6, pp. 2753-58 at p. 2753 (1987) (copy attached hereto).

The Cabilly documents do not teach or suggest applicants' realization of the benefits of using mammalian expression systems in order to effect expression of functional chimeric receptors. The Cabilly United States patent and European application contain no more than a paper reference to mammalian expression. Cabilly's statements are solely prophetic, token references, falling far short of enabling production of chimeric receptors in mammalian cells.

The Cabilly patent expressly mentions mammalian cells only once. In the detailed description of the invention, Cabilly discusses various host cell cultures. After discussing prokaryotes, eukaryotic microbes (yeast) and cell cultures from multicellular organisms, Cabilly briefly reviews possible expression control sequences and possible sources for an origin of replication for use in mammalian cells. This cursory treatment of mammalian expression is not supplemented by any other statements in the specification.

In contrast to Cabilly, applicants' specification provides a wealth of detail concerning mammalian expression.

Not only do they expressly discuss mammalian expression, but applicants go on to point out that leukocytes and myeloma cells are particularly desirable mammalian host cells (page 8, lines 18 - 23). Applicants provide further information regarding several alternatives for maintenance of tissue cultures and propagation of the cell lines (page 12, line 23 - page 13, line 2). No such information is included in Cabilly.

Transfection techniques are explored in far greater detail in applicants' specification than they were in the two sentences discussing those techniques in the Cabilly specification. In addition a citing to the calcium phosphate precipitation protocol, as did Cabilly -- a protocol that must be modified in order to obtain transfection of this kind -- applicants provide exact figures for the amounts of vectors and cells required for this specific transfection. Also in contrast to Cabilly, applicants provide a reference for the protoplast fusion technique and identify the two selection markers that were compatible with the expression system and therefore yielded successful results.

Applicants' specification includes sections on assays of antigen binding, idiotype analysis, heavy chain glycosylation and antibody production in mice after injection of transformed cells. The inclusion of these sections provides one who would try to reproduce the invention with a method for verifying the success of his work. None of these sections are included in the Cabilly specification, presumably because the bacterial cells used in Cabilly's work did not produce immunoglobulins that were glycosylated or were capable of antigen binding. Therefore, these verification tests were superfluous.

The paucity of references to mammalian expression belies the fact that all of the experimental work upon which Cabilly is based was performed in bacterial cells, namely E. coli. Each of Cabilly's Specific Examples of Preferred Embodiments (Cabilly patent, col. 16, line 63 - col. 30, line 19) involves expression only in E. coli. Consequently, detailed procedures and methods including time periods, temperatures, volumes, concentrations, etc. are available only for bacterial expression systems and no procedural details are given for mammalian systems.

Boss et al., United States patent 4,816,397, cited by the Examiner in applicants' 090,669 parent application, is not available as a prior art reference against any claim of the present application. The § 102(e) date of the Boss patent is November 14, 1984. The present application claims the benefit of the August 27, 1984 filing date of United States patent application 644,473. More specifically, the present application is a continuation of United States patent application 441,189, filed November 22, 1989 as a continuation of United States patent application 090,669, filed August 28, 1987 as a continuation-in-part of United States patent application 644,473, filed August 27, 1984. The 441,189, 090,669 and 644,473 applications are now abandoned. As compared with the specification of the 644,473 application, the additional material included in the present application disclosed and enabled intra-species chimeric receptors. The claims that rely on that additional material for support have been cancelled in this application. All the remaining claims are fully supported by the specification as originally filed in 644,473 patent application. Accordingly, the Boss patent is not prior art to any claim of this application under 35 U.S.C. § 102(e) or, consequently, under 35 U.S.C. § 103.

Pursuant to 37 C.F.R. §§ 1.56 and 1.97, applicants, through their attorneys, make of record the following documents, copies of which are submitted herewith:*

Rice et al., Proc. Natl. Acad. Sci. USA, 79, pp. 7862-65 (1983).

Axel et al., United States patent 4,399,216, "Processes For Inserting DNA Into Eucaryotic Cells And For Producing Proteinaceous Materials".

Rice has been discussed previously in this paper (supra, p.3). Axel refers to co-transforming a eukaryotic cell with DNA including a gene coding for a desired protein and with DNA including a gene coding for a selectable marker. Neither of these documents, either alone or in combination, teaches or suggests applicants' invention.

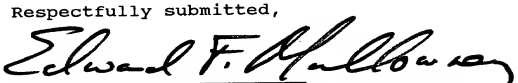
It is respectfully requested that these documents be (1) fully considered by the Examiner during the course of the examination of this application, (2) listed on the "Notice of References Cited" issued in this application and (3) printed on any patent which may issue from this application.

Having set forth the bases of patentability of the pending claims and, in accordance with § 706.07(b) of the Manual of Patent Examining Procedure (5th Edition),

* For the convenience of the Examiner, a completed Form PTO-1449, listing these documents, is attached.

applicants respectfully request that an interview with the Examiner be granted before the issuance of the first substantive Office Action in this application.

Respectfully submitted,



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Modified PTO 1083
For Other Than A Small Entity

Attorney Docket No. BD1 CIP FWC II

Applicant(s)	:	Sherie L. Morrison, et al.
Serial No.	:	07/675,106
Filed	:	March 25, 1991
For	:	RECEPTORS BY DNA SPLICING AND EXPRESSION
Group Art Unit:		185
Examiner	:	

Hon. Commissioner of Patents
and Trademarks
Washington, D.C. 20231

May 15, 1991

TRANSMITTAL LETTER

Sir:

Transmitted herewith: [ ] a Preliminary Amendment; [ ] a Response to Examiner's Action; [X] a Supplemental Amendment; [ ] a substitute Specification; [ ] a Declaration; [ ] a Supplemental Declaration; [ ] a Power of Attorney; [ ] an Associate Power of Attorney; [ ] formal drawings; to be filed in the above-identified patent application.

FEE FOR ADDITIONAL CLAIMS

[X] A fee for additional claims is not required.

[ ] A fee for additional claims is required. The additional fee has been calculated as shown below:

	CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR		PRESENT EXTRA	RATE	ADDITIONAL
TOTAL CLAIMS		-	*	=		x $20	= $
INDEPENDENT CLAIMS		-	**	=		x $60	= $
FIRST PRESENTATION OF A MULTIPLE DEPENDENT CLAIM						+ $200	= $
						TOTAL	$

* If less than 20, insert 20.
** If less than 3, insert 3.

[ ] A check in the amount of $ _____ in payment of the fee for additional claims is transmitted herewith.

[X] The Commissioner is hereby authorized to charge payment of any additional fees required under 37 C.F.R. 1.16 in connection with the paper(s) transmitted herewith, or credit any overpayment of same, to Deposit Account No. 06-1075. A duplicate copy of this transmittal letter is transmitted herewith.

[ ] Please charge $ _____ to Deposit Account No. 06-1075 in payment of the fee for additional claims. A duplicate copy of this transmittal letter is transmitted herewith.

EXTENSION FEE

[ ] The following extension fee is applicable to the Response filed herewith: [ ] $100 extension fee for response within first month pursuant to 37 C.F.R. § 1.136(a); [ ] $300 extension fee for response within second month pursuant to 37 C.F.R. § 1.136(a); [ ] $750 extension fee for response within third month pursuant to 37 C.F.R. § 1.136(a); [ ] $1,150 extension fee for response within fourth month pursuant to 37 C.F.R. § 1.136(a)

[ ] A check in the amount of [ ] $100; [ ] $300; [ ] $730; [ ] $1,150; in payment of the extension fee is transmitted herewith.

[X] The Commissioner is hereby authorized to charge payment of any additional fees required under 37 C.F.R. 1.16 in connection with the paper(s) transmitted herewith, or credit any overpayment of same, to Deposit Account No. 06-1075. A duplicate copy of this transmittal letter is transmitted herewith.

[ ] Please charge the [ ] $100; [ ] $300; [ ] $730; [ ] $1,150; extension fee to Deposit Account No. 06-1075. A duplicate copy of this transmittal letter is transmitted herewith.

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